REMARKS

Applicants have amended the specification to correct some minor typographical errors, including the paragraph bridging pages 2-3, the full paragraph on page 7, and the paragraph bridging pages 7-8. These amendments do not introduce new matter and their entry is respectfully requested.

The amendment to claim 25 is supported throughout the specification and examples. See, particularly, pages 7, 11 and 36. Claim 18 has been amended to correct an informality, namely a spelling error. Claim 25 has been amended to make explicit that which was implicit, namely that the positive and negative potentiators are cellular products; this amendment is supported by the original claim 25 and is editorial in nature. Claim 25 has also been amended to combine the steps recited in (c) and (d) into a single step, as suggested by the Examiner. In order to expedite prosecution, Applicants have canceled claims 28-40. As such, these amendments do not constitute new matter, and their entry is respectfully requested.

The Examiner has objected to Figures 3A-F, 4A-5, and 5C-D. Applicants are submitting corrected drawings along with this Amendment. Applicants have not submitted a marked-up copy of the drawings because the drawings do not contain any annotations, they are simply improved images of the previously filed drawings. Accordingly, the objection to the drawings has been obviated, and applicants respectfully request that this objection be withdrawn.

The Examiner has objected to claims 30-38, indicating that they were duplicates of claims 16-24. The Examiner has also objected to claim 39 for failing to further limit the subject matter of claim 38. Although applicants disagree, they respectfully submit that the present amendments have obviated the objections, and respectfully request their withdrawal.

The specification was objected to because of informalities, in particular spelling errors. Applicants respectfully submit that the amendments to the specification have obviated these objections, and respectfully request their withdrawal.

Claims 15-40 were rejected under 35 U.S.C. § 112, second paragraph.

Applicants respectfully submit that this rejection should be withdrawn for the following reasons.

The Examiner has indicated that claims 20 and 34 are rejected on the basis of the phrase "dominant negative mutant." Applicants respectfully submit that the phrase "dominate negative mutant" is a well-known term. As described in detail in the attached excerpt from a standard cell biology textbook, a dominant negative mutant in a diploid organism is one "in which a mutant gene eliminates the activity of its normal counterparts in the cell" (see Alberts et al., p. 326, attached hereto). A schematic of how these mutants function is provided in the figure on page 327. This excerpt also outlines a simple strategy to create such dominant negative mutations. In addition, applicants previously submitted (on August 23, 2000) the results of a Google search for this phrase, which revealed 9650 hits for that phrase, establishing the widespread acceptance as to what this term meant. The identical search performed in early December, 2004 now shows about 52,600 hits. This confirms the well known meaning and usage of this term in the art.

The use of the dominant negative mutation strategy is a broad, general approach. This approach is not limited to any specific gene, but can be widely applied. Accordingly, applicants respectfully submit that there is no need to specify any specific gene. Thus, applicants respectfully submit that the rejection of claims 20 and 34 should be withdrawn.

Applicants respectfully submit that the other rejections of claims 25 and 28 under 35 U.S.C. § 112, second paragraph have been obviated by the amendments to the claims.

Applicants have amended the claims as suggested by the Examiner, and respectfully request withdrawal of this rejection.

Accordingly, applicants respectfully submit that all claims are in compliance with 35 U.S.C. § 112, second paragraph.

Claims 15-27 and 30-39 were rejected under 35 U.S.C. § 112, first paragraph.

Applicants respectfully submit that this rejection should be withdrawn for the following reasons.

The Examiner has rejected the claims on the basis that the specification does not enable a method of selectively expressing a gene in a malignant cell, by determining whether the cell has sufficient E2F to cause increased expression of the gene. However, as taught in the specification, "selectively expressing" has a clear and specific meaning, which is different from the Examiner's reading (see e.g. pages 7-8 and the examples). The specification teaches that "selectively expressing" means that when the present method is used the gene product operably linked to the E2F responsive promoter is expressed in the malignant cell, while there is virtually no expression in the corresponding non-malignant cell.

With respect to the Examiner's objection to step (a) in claim 25, applicants respectfully submit that the specification explicitly teaches at page 11, first full paragraph, that one can readily determine if a malignant cell is an appropriate target cell by introducing an E2F vector with a marker into that cell and determining if the marker is expressed. If the marker is not expressed, it indicates that the E2F responsive promoter is being repressed. This can readily be accomplished by a simple in vitro assay based on a biopsy of tissue and applicants teach a range of factors that can be examined including the difference between the expression of a gene

product in a cancer cell versus a non-malignant dividing cell. See the specific teaching at page 36:

> These results indicate that the activity of E2F responsive promoter in tumor cells exceeds that achieved in mitotically active normal cells.

Thus, there is explicit support for determining levels of E2F expression and an example of how it can be accomplished are explicitly provided in the specification.

Claims 28, 29 and 40 were also rejected under 35 U.S.C. § 112, first paragraph.

Applicants respectfully submit that this rejection is obviated by the present amendment canceling these claims.

Accordingly, applicants respectfully submit that all claims are in compliance with 35 U.S.C. § 112, first paragraph.

Claims 15, 25, 28, 29, and 40 were rejected under 35 U.S.C. § 102(b) as being anticipated by Weintraub et al.

Applicants respectfully submit that this rejection should be withdrawn for the following reasons.

Antineoplastic agents such as e.g., radiation and chemotherapeutic agents, are not highly selective (see page 1 of the specification). For example, neither radiation nor chemotherapeutic agents distinguish between malignant and non-malignant cells. Rather they target dividing cells over non-dividing cells. Unfortunately, quiescent malignant cells are not affected while normally dividing cells are adversely affected by these therapies.

The present invention overcomes this problem by using a vector system containing an E2F responsive promoter operably linked to a gene of interest (page 7). Malignant cells express high levels of "free" E2F, which, in turn, result in the E2F responsive promoter expressing the

operably linked gene (page 8). As confirmed by the examples, the malignant cells had high levels of expression of the linked gene, whereas the normal cells displayed virtually no such expression (pages 31-35, Figures 3-5). This high selectivity between malignant and non-malignant cells was seen *in vivo*. (Ibid). Preferably, the gene of interest encodes a cytotoxic or therapeutic protein. (Pages 14-15). These genes include suicide genes such as HSV tk and toxins (page 14).

The exemplification of the present invention supports the utility of this approach for treating malignant cells that express sufficient levels of E2F. In one example, vectors having the herpes thymidine kinase (tk) gene operably linked to the E2F-1 promoter or the CMV promoter were sterotatically injected into 7 day old intracerebral C6 gliomas, followed by systemic GCV treatment for one week. Both vectors were effective in treating the tumor as treated animals lived significantly longer than control animals (page 35). However, only the vector with the E2F responsive promoter resulted in a treatment that was selective for the malignant cells. In animals with gliomas, administration of the tk gene under control of either the E2F responsive promoter or the CMV promoter resulted in extensive areas of local brain necrosis, inflammation and hemorrhage where malignant tissue was present. In normal animals, extensive areas of local brain necrosis, inflammation and hemorrhage were seen only in animals treated with the **CMV** vector, but **not** in normal animals treated with the **E2F-1** promoter. (Specification, pages 34-36). These results confirm the use of this approach to selectively target only malignant cells in a subject with a tumor, while not being expressed in the corresponding normal, non-malignant cells. This selective expression is particularly useful for the delivery of anti-cancer agents such as suicide proteins.

The results and the background supporting them are further explained by Dr. Kaelin in his Declaration submitted with the prior response. As explained therein, a gene linked to an E2F responsive promoter can be in one of three states – 1) fully repressed by a specific complex (pRB/E2F); 2) in a basal state where there is no repression; or 3) fully activated as a result of having large amounts of E2F that is not complexed with pRB, i.e., "free" E2F. The consequence for gene expression of these three different situations is in no way taught or suggested by the prior art.

Prior to the applicants' work, E2F was considered simply as an off/on trigger for gene expression. As explained by Dr. Kaelin in his Declaration (previously submitted May 30, 2003), it was known that RB negatively regulates E2F1, by binding to it and inhibiting its transactivation (Kaelin Declaration, ¶5). It was also known that their association was cell cycle regulated (Ibid). In addition, multiple groups had shown that E2F responsive promoters were more active in proliferating, non-transformed cells than in quiescent cells (Ibid ¶13). Thus, prior to the applicants present invention, the expectation would be that E2F-responsive promoters would be activated in rapidly proliferating, normal cells as well as in tumor cells (Ibid).

All that Weintraub teaches is that Rb binds to E2F and inhibits its transactivation. Weintraub pointed to the cell cycle phosphrylation/dephosphorylation of Rb, and explicitly presented a model in which E2F functions as an on/off switch:

> We therefore suggest that the E2F site alternates between a positive and negative element with the phosphorylation/dephosphorylation cycle of Rb. This cyclic activity may be responsible for activating and then inhibiting genes during the cell cycle. [Abstract; emphasis added]
>
> It would allow Rb to have a profound effect on promoter activity by switching E2F sites from positive to negative elements. [p. 261, second full paragraph; emphasis added]

Indeed, this is discussed in ¶5 of the Kaelin Declaration, in the excerpt quoted from Raj et al. The cited prior art, including both Weintraub and Raj, only taught a switching mechanism for activation by E2F as an off (i.e. bound to Rb) or on (i.e. free from Rb) switch. (Kaelin Declaration, ¶¶ 5-6).

Weintraub bases his conclusions on cell cycling stating that the Weintraub:

> "results are consistent with a model in which E2F sites in the promoters of genes important for cell proliferation are responsible for sequentially activating and inhibiting expression of these genes during the cell cycle (Fig. 4)." [p. 261, final paragraph]

Thus, based on the work of Weintraub, one would expect that in any rapidly cycling cells, E2F would activate transcription from E2F responsive promoters.

Surprisingly, applicants discovered that while expression of a heterologous gene was high in tumor cells *in vivo*, it was not in rapidly proliferating, normal hepatocytes. Applicants thus taught that this selective activation of E2F-responsive promoters in certain tumor cells could be used to selectively target tumor cells – but not normal cells. Applicants taught that to achieve this selective expression in tumor cells, one first determines whether there is a sufficient amount of E2F present in the cells to result in such a selective expression.

There is simply nothing in the prior art, including Weintraub, that in any way teaches, suggests or motivates one to look at levels of E2F expression. Weintraub is entirely focused on the association of E2F with Rb to create an on/off switch. The Examiner has pointed to Weintraub's use of several cell lines derived from carcinomas; however, these cell lines were used only because they express a mutant form of Rb, "to determine whether the inhibitory activity of the E2F site is dependent on Rb" (p. 261, lines 1-2; emphasis added).

Nothing in Weintraub teaches the present invention. Applicants have shown and Dr. Kaelin has explained that in fact what is seen in terms of results with the present invention is not just a higher level of expression that would be expected for an off/on switch, but selective expression to target expression of a toxic gene such as TK to a targeted cell type (i.e. malignant cells), without creating negative consequences from the non-selective expression of that gene in non-malignant cells.

Accordingly, applicants respectfully submit that Weintraub does not teach the present invention, and respectfully submit that this rejection of the claims should be withdrawn.

Claims 15, 16, 19-23, 25, 28-30, 33-37, and 40 were rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 94/18992 (McCormick) in view of Weintraub et al.

Applicants respectfully submit that this rejection should be withdrawn for the following reasons.

Nothing in McCormick or Weintraub or their combination in any way teaches, suggests or motivates one to look at levels of E2F expression.

The prior art does not teach or suggest a method of using an E2F responsive promoter as claimed to obtain the selective expression demonstrated. The prior art combination teaches that E2F responsive promoters respond to E2F in a *cell cycle dependent* manner (see Raj at 1286). Thus, the prior art would not have expected the selective expression systems taught herein.

As described above, Weintraub provides absolutely no basis or motivation for looking at levels of E2F and determining whether they would result in greater expression of a gene operably linked to an E2F responsive promoter than a non-malignant cell, as required by step a) of claim 25, or to look at whether a malignant cell expresses sufficient E2F to activate an E2F responsive promoter to result in expression of higher levels of a

gene operably linked to the E2F responsive promoter. McCormick adds nothing to this with respect to selectivity.

In contrast, as discussed above, Applicants taught in this specification and particularly in the Examples that the selectivity achieved and claimed herein is not a result of greater proliferation of malignant cells, but reflects a fundamental difference between a malignant cell and a non-malignant cell. This selectivity is in no way taught.

The Examiner has acknowledged that McCormick does not teach a method of determining whether a malignant cell expresses enough E2F to allow selective activation of an E2F responsive promoter compared to a mitotically active, normal cell.

Further, even if the references were combinable, such combination would not suggest the claimed invention. There is nothing in any of these references that suggests such a selective expression. Thus, there is no suggestion and no reasonable expectation of success in any of the cited references for selective expression in malignant cells of genes under the control of an E2F responsive promoter.

Accordingly, Applicants respectfully submit that this rejection of the claims should be withdrawn.

Claims 15, 16, 19-30, 33-38, and 40 were rejected pursuant to 35 U.S.C. §103(a) as being unpatentable over McCormick in view of Weintraub et al. further in view of US patent 6,310,045 (the '045 patent).

Applicants respectfully submit that this rejection should be withdrawn for the following reasons.

The addition of the '045 patent does not overcome the fundamental deficiency of the combination of McCormick in view of Weintraub, described above. The '045 is

directed to expressing genes in a solid tumor cell. One of the genes described is the gene encoding Pseudomonas exotoxin A. However, the '045 in no way suggests the present invention, because it provides no suggestion to selectively express genes in malignant cells by taking first determining whether the malignant cells express sufficient levels of E2F. This fundamental deficiency of Weintraub and McCormick is in no way cured by the specific genes taught by the '045.

Claims 16-18, 25, and 26 were rejected pursuant to 35 U.S.C. §103(a) as being unpatentable over McCormick in view of Weintraub et al. further in view of Raj et al.

Applicants respectfully submit that this rejection should be withdrawn for the following reasons.

Applicants respectfully submit that a fair reading of Raj shows that E2F was relatively unimportant, and worked in a cell cycle dependent manner (see page 1286). Additionally, Raj taught that expression of anti-sense E2F1 showed no significant effect on transcriptional activities of the test promoters. And, specifically Raj concludes that there is a functionally distinct complex i.e. GEAP, that is negatively influenced by E2F1. The discussion with respect to Raj, when read in its entirety, is that E2F and E2F responsive promoters interact in a cell-cycle dependent manner and would thus indicate that there should be no difference between its effect in normal proliferating cells and malignant cells. Thus, the addition of Raj adds nothing to the combination of Weintraub and McCormick to teach or suggest the present invention.

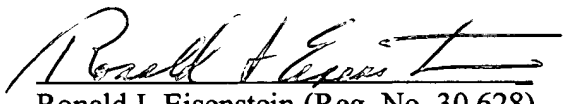
Accordingly, Applicants respectfully submit that this rejection of the claims should be withdrawn.

In view of the foregoing, Applicants respectfully submit that all claims are in condition for allowance. Early and favorable action is requested.

If any additional fee is required, please charge Deposit Account No. 50-0850.

Date: December 13, 2004

Respectfully submitted,

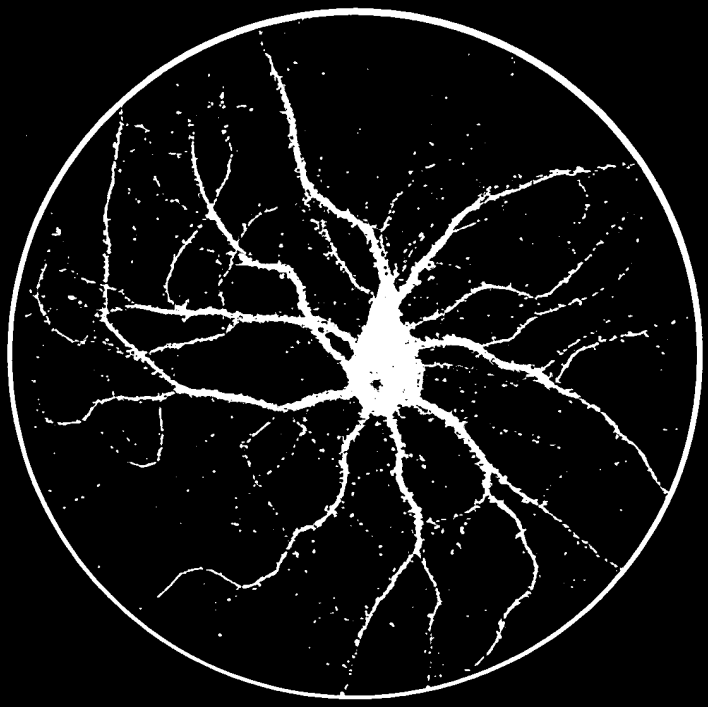


Ronald I. Eisenstein (Reg. No. 30,628)
Nicole L. M. Valtz (Ref. No. 47,150)
NIXON PEABODY LLP
100 Summer Street
Boston, MA 02110
Tel: (617) 345-6054
Fax: (617) 345-1300

EXHIBIT 1

MOLECULAR BIOLOGY OF THE CELL

THIRD EDITION



Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff
Keith Roberts • James D. Watson

GARLAND STAFF
Text Editor: Miranda Robertson
Managing Editor: Ruth Adams
Illustrator: Nigel Orme
Molecular Model Drawings: Kate Hesketh-Moore
Director of Electronic Publishing: John M-Roblin
Computer Specialist: Chuck Bartelt
Disk Preparation: Carol Winter
Copy Editor: Shirley M. Cobert
Production Editor: Douglas Goertzen
Production Coordinator: Perry Bessas
Indexer: Maija Hinkle

Bruce Alberts received his Ph.D. from Harvard University and is currently President of the National Academy of Sciences and Professor of Biochemistry and Biophysics at the University of California, San Francisco. *Dennis Bray* received his Ph.D. from the Massachusetts Institute of Technology and is currently a Medical Research Council Fellow in the Department of Zoology, University of Cambridge. *Julian Lewis* received his D.Phil. from the University of Oxford and is currently a Senior Scientist in the Imperial Cancer Research Fund Developmental Biology Unit, University of Oxford. *Martin Raff* received his M.D. from McGill University and is currently a Professor in the MRC Laboratory for Molecular Cell Biology and the Biology Department, University College, London. *Keith Roberts* received his Ph.D. from the University of Cambridge and is currently Head of the Department of Cell Biology, the John Innes Institute, Norwich. *James D. Watson* received his Ph.D. from Indiana University and is currently Director of the Cold Spring Harbor Laboratory. He is the author of *Molecular Biology of the Gene* and, with Francis Crick and Maurice Wilkins, won the Nobel Prize in Medicine and Physiology in 1962.

Library of Congress Cataloging-in-Publication Data
Molecular biology of the cell / Bruce Alberts . . . [et al.].—3rd ed.
p. cm.
Includes bibliographical references and index.
ISBN 0-8153-1619-4 (hard cover).—ISBN 0-8153-1620-8 (pbk.)
1. Cytology. 2. Molecular biology. I. Alberts, Bruce.
[DNLM: 1. Cells. 2. Molecular Biology. QH 581.2 M718 1994]
QH581.2.M64 1994
574.87—dc20
DNLM/DLC
for Library of Congress 93-45907
CIP

Published by Garland Publishing, Inc.
717 Fifth Avenue, New York, NY 10022

Printed in the United States of America

15 14 13 12 10 9 8 7 6 5 4 3 2 1

Front cover: The photograph shows a rat nerve cell in culture. It is labeled with a fluorescent antibody that stains its cell body and dendritic processes (*yellow*). Nerve terminals (*green*) from other neurons (not visible), which have made synapses on the cell, are labeled with a different antibody. (Courtesy of Olaf Mundigl and Pietro de Camilli.)

Dedication page: Gavin Borden, late president of Garland Publishing, weathered in during his mid-1980s climb near Mount McKinley with MBoC author Bruce Alberts and famous mountaineer guide Mugs Stump (1940–1992).

Back cover: The authors, in alphabetical order, crossing Abbey Road in London on their way to lunch. Much of this third edition was written in a house just around the corner. (Photograph by Richard Olivier.)

but is abnormal in the process for which the gene is required. Often the mutant of choice is one that produces a temperature-sensitive gene product, which functions normally at one temperature but is inactivated when cells are shifted to a higher or lower temperature.

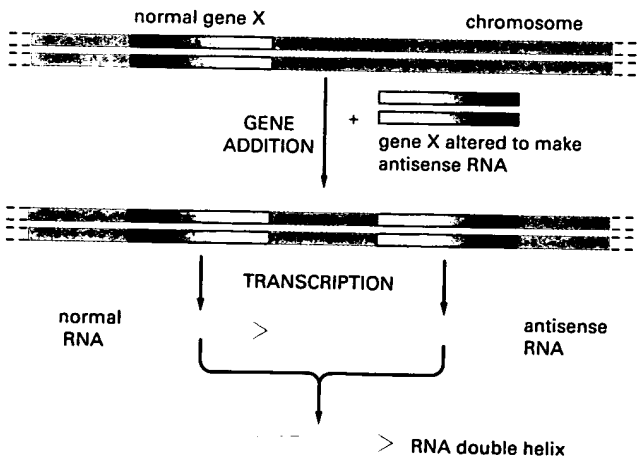
The ability to perform direct *gene replacements* in lower eucaryotes, combined with the power of standard genetic analyses in these haploid organisms, in large part explains why studies in these types of cells have been so important for working out the details of those processes that are shared by all eucaryotes. Gene replacements occur more rarely in higher eucaryotes, for reasons that are not known.

Engineered Genes Can Be Used to Create Specific Dominant Mutations in Diploid Organisms [36]

Higher eucaryotes, such as mammals or fruit flies, are diploid and therefore have two copies of each chromosome. Moreover, transfection with an altered gene generally leads to *gene addition* rather than gene replacement: the altered gene inserts at a random location in the genome, so that the cell (or the organism) ends up with the mutated gene in addition to its normal gene copies (Figure 7–42B).

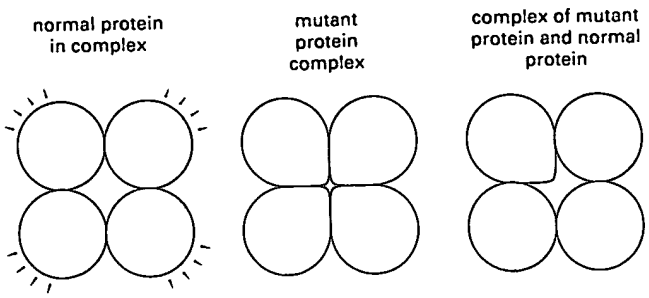
Because gene addition is much more easily accomplished than gene replacement in higher eucaryotic cells, it would be enormously useful to be able to create specific **dominant negative mutations** in which a mutant gene eliminates the activity of its normal counterparts in the cell. One ingenious and promising approach exploits the specificity of hybridization reactions between two complementary nucleic acid chains. Normally, only one of the two DNA strands in a given portion of double helix is transcribed into RNA, and it is always the same strand for a given gene. If a cloned gene is engineered so that the opposite DNA strand is transcribed instead, it will produce **antisense RNA molecules** that have a sequence complementary to the normal RNA transcripts. Antisense RNA, when synthesized in large enough amounts, will often hybridize with the "sense" RNA made by the normal genes and thereby inhibit the synthesis of the corresponding protein (Figure 7–43). A related method is to synthesize short antisense nucleic acid molecules by chemical or enzymatic means and then inject (or otherwise deliver) them into cells, again blocking (though only temporarily) production of the corresponding protein.

For unknown reasons the antisense RNA approach frequently fails to inactivate the desired gene. An alternative way of producing a dominant negative mutation takes advantage of the fact that most proteins function as part of a



FORMATION OF RNA/RNA HELIX PREVENTS THE SYNTHESIS OF A PROTEIN PRODUCT FROM NORMAL GENE X

Figure 7–43 **The antisense RNA strategy for generating dominant negative mutations.** Mutant gene that have been engineered to pr antisense RNA, which is complementary in sequence to th RNA made by the normal gene X. cause double-stranded RNA to fo inside cells. If a large excess of th antisense RNA is produced, it can hybridize with—and thereby inactivate—most of the normal RN produced by gene X. Although in t future it may become possible to inactivate any gene in this way, at present the technique seems to wo for some genes but not others.



A dominant negative effect of a protein. Here a gene is engineered to produce a mutant protein that prevents the normal copies of the same protein from performing their function. In this simple example the normal protein must form a multi-subunit complex to be active, and the mutant protein blocks function by forming a mixed complex that is inactive. In this way a single copy of a mutant gene located anywhere in the genome can inactivate the normal products produced by other gene copies.

protein complex. Such complexes can be inactivated by the inclusion of one nonfunctional component. Therefore, by designing a gene that produces quantities of a mutant protein that is inactive but still able to assemble into complex, it is often possible to produce a cell in which all the complexes are activated despite the presence of both normal and mutant copies of the protein (Figure 7–44).

If a protein is required for the survival of the cell (or the organism), a dominant negative mutant will die, making it impossible to test the function of the protein. To avoid this problem, one can couple the mutant gene to control sequences that have been engineered to produce the gene product only on command –for example, in response to an increase in temperature or to the presence of a specific signaling molecule. Cells or organisms containing such an *inducible* dominant mutant gene can be deprived of a specific protein at a particular time, and the effect can then be followed. In the future, techniques for producing dominant negative mutations to inactivate specific genes are likely to be widely used to determine the functions of proteins in higher organisms.

Engineered Genes Can Be Permanently Inserted into the Germ Line of Mice or Fruit Flies to Produce Transgenic Animals [37]

The ultimate test of the function of an altered gene is to reinsert it into an organism and see what effect it has. Ideally one would like to be able to replace the normal gene with the altered one so that the function of the mutant protein can be analyzed in the absence of the normal protein. As discussed above, this can be readily accomplished in some haploid organisms, but in higher eucaryotic cells an integrative event leading to a gene replacement occurs only very rarely. Foreign DNA can, however, rather easily be randomly integrated into the genome. In mammals, for example, linear DNA fragments introduced into cells are rapidly ligated end to end by intracellular enzymes to form long tandem arrays, which usually become integrated into a chromosome at an apparently random site. Fertilized mammalian eggs behave like other mammalian cells in this respect. A mouse egg injected with 200 copies of a linear DNA molecule will often develop into a mouse containing, in many of its cells, a tandem array of copies of the injected gene integrated at a single random site in one of its chromosomes (Figure 7–45). If the modified chromosome is present in the germ line cells (eggs or sperm), the mouse will pass these foreign genes on to its progeny. Animals that have been permanently altered in this way are called **transgenic organisms,** and the foreign genes are called **transgenes.** Because the normal gene generally remains present, only dominant effects of the alteration will show up. Nevertheless, such transgenic animals have already provided important insights into how mammalian genes are regulated and how certain altered genes (called oncogenes) cause cancer.

It is also possible to produce transgenic fruit flies, in which single copies of genes are inserted at random into the *Drosophila* genome. The trick in this case is to insert the DNA fragment between the two terminal sequences of a par-